## REMARKS

In this response and amendment, claim 1 has been amended. No claims have been canceled or added. Claim 21 has been withdrawn by Examiner. Accordingly, claim 1 remains pending in the present application.

I. Election/Restriction

On page 2 of the Office Action, Examiner recites in part:

"...newly submitted claim 21 directed to an invention that is independent or distinct from the invention originally claimed . . . newly submitted claim 21 is drawn to an independent diagnostic method which can be achieved by performing a biopsy, using a regular or fluorescence microscopy. . . . Instant claims are based on a diagnostic method that can be achieved visually by using a visual light. Since applicant has received an action on the merits for the originally presented invention, this invention has been constructively elected by original presentation for prosecution on the merits. Accordingly, claim 21 is withdrawn from consideration as being elected to a non-elected invention."

In view of the foregoing, Applicant reserves the right to pursue the subject matter of claim 21 in a divisional and/or continuation application.

II. Claim Rejections - 35 U.S.C. § 112

On page 3 of the Office Action, claim 1 was rejected under 35 U.S.C. 112, first paragraph, for failing to comply with the written description requirement. Specifically, the Examiner recites in relevant part:

". . . Applicant claims 'detecting by visible light during visual examination the retention of said agent by the mitochondria of cancer cells in vivo.' The specification does not explain how the step of visual examination that would make the retention of the stain visible by visual examination possible since mitochondria is a subcellular structure which cannot be seen without an electronic microscope. Thus the specification does not convey to a person having ordinary skill in the art to envisage how the method of visually detecting the retention of the agent by the mitochondria could be achieved."

Applicant respectfully disagrees. However, in order to expedite prosecution of the application, Applicant has amended claim 1 to recite in relevant part: ". . . detecting by a microscope under visible light during visual examination. . ." This amendment is fully supported by the specification as filed and is not new matter. For example, on page 12 of the specification as filed, it recites: "After incubation and rinsing of various cell lines, using different cationic marking agents, the mitochondrial localization of the agents is analyzed using confocal high

resolution microscopy and phase contrast microscopy." Further, it recites on page 14 of the specification as filed: "Fresh explants of resected epithelial carcinomas are analyzed for marking agent uptake and retention. After resection, the carcinomas are microdissected from surrounding tissue, cut into 3 mm sections . . ." Furthermore, Applicant submits that one skilled in the art knows how to take these resected sections, put them on a microscope slide, prep them for viewing under a microscope, etc. Additionally, Applicant submits that one skilled in the art would know that agent retention in the mitochondria can be viewed using a microscope. Therefore, withdrawal of the rejection under 35 U.S.C. 112, first paragraph is respectfully requested.

Furthermore, claim 1 was rejected under 35 U.S.C. 112 second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as her invention. Specifically, Examiner recites on pages 3-4 of the Office Action:

"The claim recites 'detecting by visible light during visible examination the retention of said agent by the mitochondria of cancer cells in vivo.' The mitochondria

cannot be seen under visible light during visual examination. The mitochondria cannot be seen without a microscope. Thus the claim is not clear of how to detect the mitochondria during visual examination."

Applicant respectfully disagrees. However, to expedite prosecution of the application, Applicant has herein amended claim 1 to recite in relevant part: "detecting by a microscope under visible light. . ." Accordingly, the mitochondria can be seen under visible light, using a microscope. This is opposed to a fluorescent dye, which cannot be seen under visible light using an ordinary microscope. Fluorescent dyes can be viewed, for example, using a fluorescence microscope.

Therefore, withdrawal of the rejection under 35 U.S.C. 112, second paragraph is respectfully requested.

III. Claim Rejections - 35 U.S.C. 103

On page 4 of the Office Action, Claim 1 was rejected under 35 U.S.C. 103(a) as being unpatentable over Pomerantz ("Pomerantz") (WO 9726018) in view of Oseroff et al., (Intramitochondrial Dyes Allow Selective in vitro Photolysis of Carcinoma Cells, PNAS, December 15, 1986, vol. 83, no.24, 9729-9733 ("Oseroff") and further view of

Brenner, S. et al., Supravital staining of mitochondria with phenosafranin dyes, Biochem et Biophys. 1953, pages 11480-11486. ("Brenner").

On pages 4-6, the Office Action recites in part:

"The reference teaches in vivo detection (corresponds to step b of claim 1) of oral premalignant lesions and oral carcinomas . . . Pomerantz also disclosed that this type of staining is dependent on the dye gaining access to internal subcellular structures such as the nucleus. Such access is readily obtained only by 'fixing' a tissue sample of formaldehyde or other reagent that disrupts the cellular membrane without destroying general cellular structure (page 2, line 26 bridging to page 3, line 4). Note that it is expected that the mitochondria as a subcellular structure would at least partially absorb the dye . . . Pomerantz did not disclose the specific retaining of the mitochondria to the dyes recited in instant claim 1 as amended."

Applicant respectfully disagrees. Pomerantz teaches that dyes may cross the cellular membrane when they are fixed by agents known to disrupt the membrane; however, it does not necessarily hold that because of this, "the mitochondria as a subcellular structure would at least partially absorb the dye." Indeed, the Examiner recognizes that "Pomerantz did not disclose the specific retaining of the mitochondria to the dyes recited in instant claim 1 as amended" (Office Action, page 5). As one skilled in the art knows, absorption and/or the travel (or crossing) of

molecules across various membranes is a complex biochemical process based upon a variety of factors, such as the structure of the molecule, etc. As such, Applicant submits that Pomerantz does not teach nor suggest that the dye crosses the mitochondrial membrane.

Furthermore, on page 5 of the Office Action, Examiner recites:

"Oseroff teaches that carcinoma cell mitochondria preferentially accumulate and retain cationic dyes to a much greater extent that most normal cells. In addition, Oseroff teaches that rhodamine and cyanine dyes were tested because they potentially serve as targets for highly selective photochemotherapy. It is noted that alcian blue dye recited by Applicant in amended claim 1 is a cationic dye. Oseroff also teaches. . . that cationic dyes can preferentially accumulate within carcinoma cells and demonstrates that highly selective, light-induced mitochondrial damage and cell killing are possible after brief exposure to some of these dyes. An important aspect of this work is that photolysis depends only on two relatively 'generic' properties mitochondrial accumulation and photosensitization. Thus, though EDKC was the most selective phototoxin in the group of dyes that we evaluated, it is possible that other cationic molecules that concentrate within the mitochondria at higher levels or that is more efficient photosensitizers will be still more effective . . . Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to use a non-toxic dye other than toluidine blue O to mark premalignant carcinoma cells as Pomerantz teaches and Oseroff teaches that mitochondria and carcinomatous cells can absorb cationic dyes to a much greater extent than normal cells . . ."

The Examiner alleges that since Oseroff teaches the

generic concept of using cationic dyes, "it is possible that other cationic molecules that concentrate within the mitochondria" would be obvious to one skilled in the art as well.

Applicant respectfully disagrees and submits that Examiner has not met her burden in establishing the *prima facie* case of obviousness. Applicant submits that "when a single prior art reference which discloses a genus encompassing the claimed species or subgenus but does not expressly disclose the particular claimed species or subgenus, Office personnel should attempt to find additional prior art to show that the differences between the prior art primary reference and the claimed invention as a whole would have been obvious. Where such additional prior art is not found, Office personnel should consider the factors discussed below to determine whether a single reference 35 U.S.C. 103 rejection would be appropriate." M.P.E.P. 2144.08. Here, Applicant submits that Examiner simply alleges that since the dyes as recited in claim 1 are cationic, they are obvious over Oseroff (and Pomerantz/Brenner), as it would be obvious to try using different cationic dyes to come up with the claimed invention. Indeed, Examiner recites on page 5 of the Office

Action that ". . . it is possible that other cationic molecules that concentrate within the mitochondria at higher levels . . . will still be more effective." However, Examiner has not delineated a prima facie case of obviousness as required by M.P.E.P. 2144.08. The fact that a claimed species or subgenus is encompassed by the prior art genus (i.e., a mere statement that a dye is in the group of cationic dyes and therefore should behave in a like manner) is not sufficient by itself to establish a *prima facie* case of obviousness. M.P.E.P. 2144.08. Additionally, a mere *possibility* that other cationic dyes may be effective is not sufficient to establish a *prima facie* case for obviousness. Accordingly, Applicant submits that Examiner has not met her burden in establishing the *prima facie* case.

For example, Applicant submits that it is not only the cationic nature of the dye that allows it to penetrate the mitochondria. For example, described on pages 9 and 10 of the specification are various mechanisms of entry into the mitochondria. Entry is affected by (1) the availability of the positive charge (i.e., as one skilled in the art knows, molecules could be folded and/or certain elements on the molecule could be masked, such as the positive charge

itself); (2) the structure (and therefore identity) of the molecule (not simply that it is cationic) but other species/chemical groups on the molecule may bind to specific active sites, i.e., specific proteins, on the mitochondria; i.e., one skilled in the art knows that selective binding is not simply contingent upon charge; and (3) the hydrophobic/hydrophilic nature of the dye (once again, one skilled in the art knows that this is based upon the identity of the dye).

Furthermore, Applicant submits that neither Pomerantz nor Oseroff teach nor suggest the claim element ". . . the retention of said agent by the mitochondria of cancer cells ***in vivo*** which have been marked by absorption of said agent in the mitochondria thereof . . ." as presently claimed. Pomerantz teaches that the dye may cross the cellular membrane once the cells are fixed. One skilled in the art knows that this is *in vitro* retention (or uptake), not *in vivo* retention.

In addition, Oseroff does not teach nor suggest *in vivo* retention of the dye. For example, it recites, "This report demonstrates the feasibility of selective carcinoma cell photolysis (SCCP) *in vitro*, for human squamous,

bladder, and colon carcinoma cells, using long-wavelength absorbing dyes." Additionally, the Examiner alleges that "Oseroff teaches that carcinoma cell mitochondria preferentially accumulate and retain cationic dyes to a much greater extent than most carcinoma cells . . ." Applicant notes that Oseroff tests the following cancer cell lines: EJ bladder carcinoma cells, CX-1 (human adenocarcinoma cell line), and the FaDu cell line (upper respiratory tract tumor - squamous cell line). As one skilled in the art knows, absorption and/or retention of various cationic dyes vary depending on cell type. For example, Table 1, p. 9731 of Oseroff itself shows differences in the intracellular uptake of EDKC depending on cell line type. Accordingly, simply because EKDC shows some sort of a mitochondrial uptake in the (3) cancerous cell lines studied in Oseroff (however highly varied), does not mean that the dyes recited in claim 1 will exhibit the same or similar uptake in oral epithelial cells. Uptake varies depending on cell type as Oseroff itself teaches.

In addition, claim 1 recites, in relevant part: "***…contacting said oral epithelium containing cancerous cells in the locus of normal cells*** with one or more

agents. . ." Neither Pomerantz nor Oseroff teaches nor suggests the foregoing. Oseroff compares the retention and photolytic ability of, for example, EDKC in two different cell lines, one being a carcinogenic cell line, and the other being a non-carcinogenic cell line. For example, in Oseroff, the EJ cell line is contacted with EDKC (in one petri dish, for example). The CV-1 cell line (or the human keratinocyte cell line, a non-cancerous cell line) is contacted with EDKC (in a second petri dish, for example). As such, Oseroff does not teach or suggest "in the locus of normal cells" as presently claimed. For example, on page 9731 it recites: "EDKC showed a remarkable difference in phototoxicity between EJ and CV-1 cells (human keratinocyte cell line, known in the art as a non-cancerous cell line), as shown qualitatively in the photomicrographs depicting the boundary zone (without light/with light) for each cell type (Fig. 3). EJ cells exposed to the dye alone (Fig. 3, left, lower half) were essentially unaffected, whereas addition of light eliminated almost all of the cells. In contrast, the CV-1 cells on either side of the boundary appear identical. Differences in phototoxicity among the carcinoma cell lines correlated with the uptake of EDKC (Table 1)

Accordingly, Applicant submits that the pending claim is not obvious over Pomerantz in view of Oseroff (and in further view of Brenner as discussed below).

On page 6 of the Office Action, it recites in relevant part:

"Brenner teaches that phenosafranin dyes supravitally stains mitochondria (title). The reference teaches that the possibility must be considered that while many basic dyes may absorb onto isolated mitochondria all do not permeate with equal facility into the living cell. Weakly basic dyes like neutral red penetrate the cell membrane as undissociated bases and stain the vacuoles forming coacervates with ribonucleoprotein. The phenosafranin dyes, however, are completely ionized at neutral pH and would have to penetrate as dye cations (485)...It would have been obvious to a person of having ordinary skill in the art at the time the invention was made to include phenosafranin in a trial to detect early dysplasia of oral carcinomas because Oseroff motivated people of ordinary skill in the art to try different cationic non-toxic dyes for early detection of cancers. The person of ordinary skill would use the steps suggested by Pomerantz since the reference teaches topical easy method of early detecting oral cancers. The expected result would be a method to detect cancerous cells of the oral epithelium in vivo using cationic non-toxic dyes such as phenosafranin and alcian blue."

Applicant respectfully disagrees. Brenner recites, for example, on page 480: "Although Janus Green B is widely used as a supravital stain for mitochondria there is no adequate theory to explain the mechanism of action. Janus green B is diethylphenosafraninaxzodimethylaniline and

Cowdry demonstrated that diethylsafranin itself, as well as other dyes containing it, stained mitochondria supravitally. Because dimethylphenosafranin and its derivatives failed to give the selective supravital staining reaction, Cowdry claimed that the specificity of Janus green B depends on the diethylphenosafranin part of the molecule . . . akyl substitution on an amino group attached to the aromatic ring increases the positivity of the nitrogen atom and ethyl groups exert a stronger effect than methyl groups. Thus the diethylphenosafranins would be expected to be more basic than the dimethylphenosafranins and this difference between the two types of dyes might be related to the staining specificity. To test this hypothesis, a series of symmetrical and unsymmetrical phenosafranin dyes were investigated for their supravital staining activity." The results of the investigation in Brenner are outlined in Table II on page 482. Table II shows that phenosafranin ***does not*** stain mitochondria. Additionally, it recites on page 485: ". . . In such preparations, Janus green B potently inhibits oxidative phosphorylation, but this property is not shared by phenosafranin and other dyes which are not supravital mitochondrial stains. . .". Accordingly, Brenner teaches that phenosafranin does not stain mitochondria, contrary to

what is claimed. It would not be obvious to try using phenosafranin as an agent that stains and/or is retained by the mitochondria. Accordingly, Applicant submits that it would not have been obvious to try using phenosafranin as a supravital mitochondrial staining agent, as Brenner teaches that it *is not* a supravital mitochondrial marking agent, ***contrary*** to what is claimed.

On pages 6 and 7, Examiner recited her response to arguments presented in the last office action response. Applicant respectfully disagrees. However, in order to expedite prosecution of the application, Applicant has presented additional arguments herein.

Accordingly, withdrawal of the rejection under 35 U.S.C. 103 is respectfully requested.

## CONCLUSION

For the reasons noted above, Applicant respectfully requests that the rejection of the claim be reconsidered and withdrawn, and that claim 1 pending in this application, be allowed. It is believed that the claim now pending patentably defines the subject invention over the prior art of record and is in condition for allowance and such action is earnestly solicited at the earliest possible date.

The Commissioner is hereby authorized to charge any additional fees necessary to Deposit Account 10-0440, or to credit any overpayment to the same.

Respectfully submitted,

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